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"RADIOMETRIC METHODS FOR RAPID DIAGNOSIS OF VIRAL INFECTION"

Combined Quarterly Report

Steven M. Larsen, M.D.

November 15, 1974

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

A rapid radiometric technique was developed for detecting the presence of herpes simplex virus type 1 in stationary monolayers of the diploid cell line WI-38. The time of detection was compared to that obtained from visual examinations for cytopathic effects in the same cell line. Glucose-1-^{14}-C oxidation of infected and uninfected cells was determined by $^{14}CO_2$ production by the ionization chamber, Bactec R-301. Infected cells showed a 23-26%

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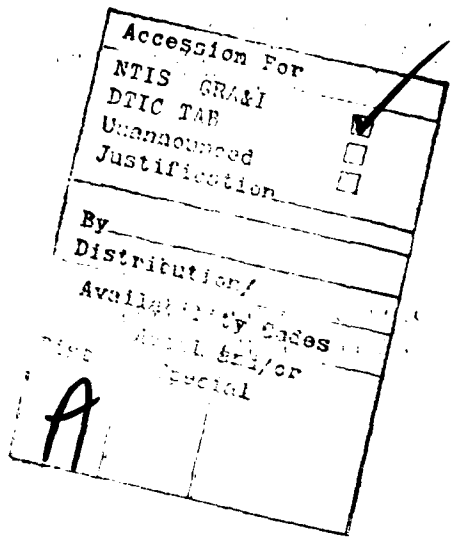
reduction in glucose-1-^{14}-C oxidation 4-6 hours post-infection, as compared to uninfected control cells. This change in cellular metabolism was observed 14 hours before visible signs of cytopathic effect. Specific antiserum will be used to neutralize the viral effect for the purpose of speciation. The radiometric method for the detection of viral effect on host metabolism is simple, objective, and deserves further investigation.



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The specific aim of this contract work was to develop a radiometric testing system for rapidly determining the effect of virus on cellular metabolism, as the basis for rapid diagnosis of viral infections. There are two distinct elements to this work - the detection of virus by its effect on cellular metabolism and the identification of the particular virus causing this effect.

Virus Detection

Much of our initial work has been directed toward development of a suitable tissue culture test system. To date, we have had most extensive experience with a test system which measures the effect of Herpes simplex type 2 virus on (Wi 38)tissue culture cells. To develop this system, we evaluated Wi 38 and Hep 2 cells; concentration of glucose in the tissue culture media; position of labeling of the ^{14}C-glucose substrate; concentration of stable CO_2 in this atmosphere; optimal buffer system. We have sought to optimize the early release of $^{14}CO_2$ to make detection of viral effect as rapid as possible.

Our current test system consists of Wi 38 tissue culture monolayers maintained in low glucose medium (Minimal essential media, Eagle; Earl's base) plus streptomycin 400 µg/ml; kariamycin 130 µg/ml; penicillin 400 µU/ml plus glutamine and 3% calf serum. 1 µCi of (1,^{14}C)-glucose is used as the radioactive substrate, and the cellular metabolism is measured using a radiometric detection system (Bactec R-301) which is based on an ion chamber device.

A relatively heavy inoculum of virus was used for these studies, which was obtained from frozen Wi 38 virus mixture with 4+ cytopathic effect (CPE). This preparation had approximately 2×10^5 plaque forming units per ml.

At the start of the test, the media was withdrawn and 0.1 cc or about 2×10^4 PFU of viral inoculum was added and incubated at 37°C for 90 minutes with agitation every 15 minutes. 1 µCi of substrate was then added to the vials. The low glucose media was then added to a total volume of 2 cc.

The metabolism of the tissue culture cells was measured at 4-6 hours, 24 hours, and 48 hours and all points were run in quintiplicate. A significant effect was observed by 4 hours with depression of the metabolism in the virus infected cells. This was at least 20 hours before the earliest indication of CPE which was also evaluated in parallel. The preliminary work on the test system is quite encouraging and reproducible. The detection phase for Herpes simplex is well enough worked out to proceed to the identification phase.

Identification

Antisera to virus will be used to identify the particular virus responsible for the altered cellular metabolism by specifically neutralizing the virus effect.

To date, we have run several experiments using pooled human serum as the anti-Herpes simplex type 2 neutralizing substance. We have been able to demonstrate a neutralizing effect of the virus antisera as measured by enhanced metabolism in one culture containing viral antisera + virus as compared to controls with virus alone.

Future Work

Further work on the identification phase of this work is necessary. One problem is that the antisera alone contains substances which decrease production of $^{14}CO_2$ glucose by the tissue culture system. This may be due to toxic substances or most likely simple competition between stable glucose in the antisera and radioactive glucose in the media. We plan to remove the glucose by dialysis from the antisera to virus, and make further purification if necessary. Our goal is to obtain a detection system which is able to detect and identify virus within 3-4 hours. This goal seems to be within reach.

Not all viruses are amenable to study by effect on tissue culture systems and for this reason we have begun to explore an alternative technique - Utilization of specific antisera to promote $^{14}CO_2$ release from the metabolism of (1-^{14}C) glucose during phagocytosis by polymorphonuclear leukocytes. We are just beginning this work, but we have shown specific effect of antisera to influenza type A $^{14}CO_2$ release during phagocytosis. We plan to use antisera against influenza A, B and C and the killed A, B, C virus, to evaluate the potential for specific detection using the phagocytosis system.

Sincerely,

Steven M. Larson, M.D.

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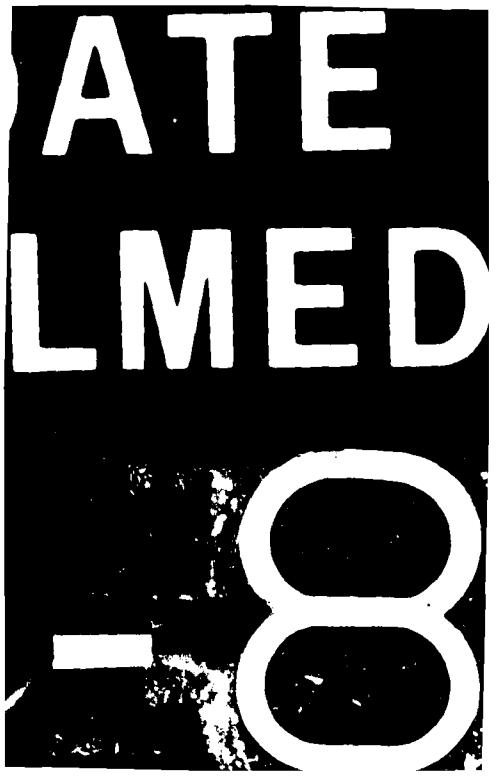
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